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Site-Selective DNA cleavage by a novel complex of copper-conjugate of Phen and polyamide containing *N*-methylimidazole rings

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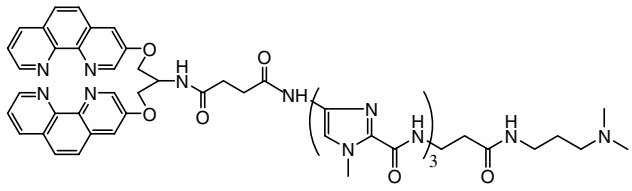
Abstract—A novel conjugate of 3-Clip-Phen and polyamide containing three *N*-methylimidazole (Im) rings was synthesized for the targeting human telomeric repeat of 5′-TTAGGG-3′, and the DNA cleaving activity and the sequence selectivity of the complex of copper-conjugate were confirmed by electrospray ionization mass spectrometry.

Human telomeric DNA consisting of tandem repeats of 5′-TTAGGG-3′ for many kilobases is essential for chromosomal stability.[1–3] Recent research reported that telomere and telomerase have obvious correlation with human tumors.[4,5] Therefore, the telomere repeat sequence (5′-TTAGGG-3′) is an important target for new antitumor drugs.

The DNA cleavage agents have also generated considerable attention in chemistry, biology, and medicine.[6–8] Among these agents, $Cu(Phen)_2$ complex can efficiently cleave double-stranded DNA in the minor groove with mercaptopropionic acid (MPA) as a co-reactant.[9–14] In the past decade, some DNA cleavage agents conjugated with DNA binding groups, such as polyamides containing *N*-methylpyrrole, have been synthesized for selectively binding the A·T-rich sequence of DNA.[15,16] In order to confirm the cleaving sites and the oxidation mechanism, the DNA cleaving activities were always analyzed by the PAGE with complicate and long time cost procedures.

In this paper, 5′-$(TTAGGG)_2$-3′/5′-$(CCCTAA)_2$-3′, telomeric DNA sequence, was selected as target, and a novel conjugate (3-Clip-Phen-Im_3-β-Dp) was synthesized (Scheme 1), which was expected to bind G·C rich sequence.[17,18] To avoid the complicated procedures of the PAGE, the ESI-MS, a convenience analysis, was innovatively utilized to investigate site-selective DNA cleavage with this novel complex.

First two 3-Br-Phen molecules were linked with serinol to give the compound of 3-Clip-Phen (Scheme 2), and the amino group of 3-Clip-Phen directly reacted with succinic anhydride to produce 3-Clip-Phen-COOH.[19] NO_2ImImImβDp was synthesized by the convenient haloform reaction and the DCC (dicyclohexylcarbodiimide)/HOBt (1-hydroxybenzo-triazole) coupling reaction.[20] Then, 3-Clip-Phen-COOH were linked with this polyamide by the hydrogenation and the coupling reaction in the solution phase to give 3-Clip-Phen-Im_3-β-Dp. This conjugate was coordinated with 1 equiv of



Scheme 1. Structures of 3-Clip-Phen-Im_3-β-Dp.

Keywords: DNA cleaving activity; ESI-mass spectrometry; Site-selective DNA cleavage.
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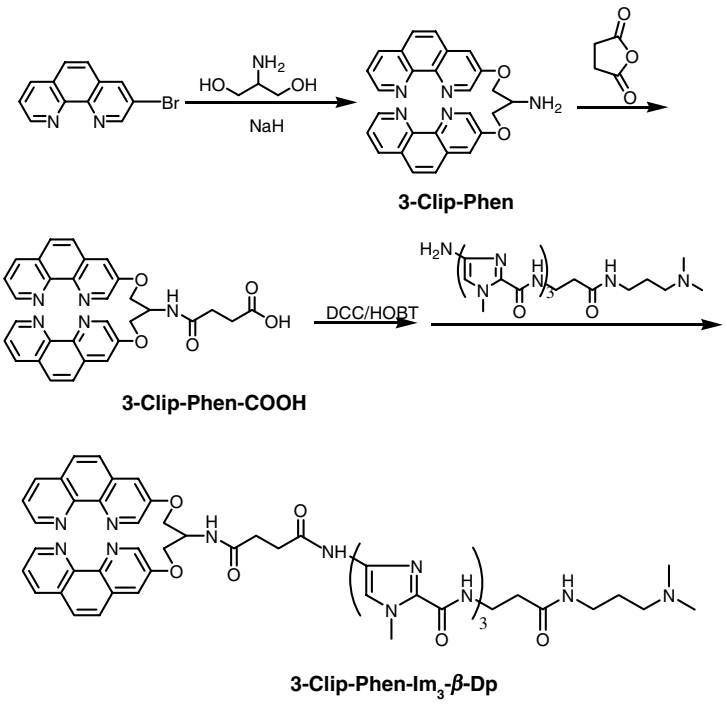


Scheme 2. Synthetic routes of 3-Clip-Phen-Im_3-β-Dp.

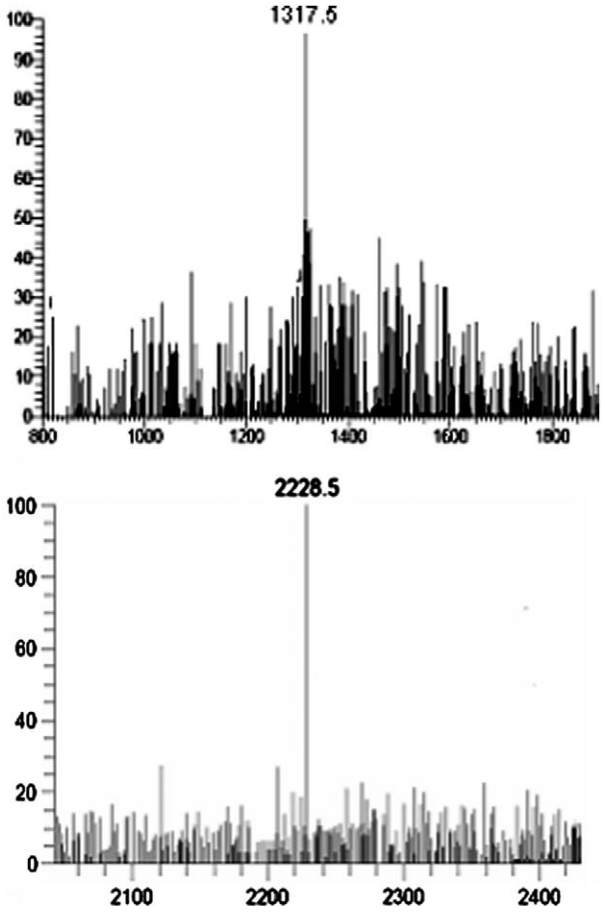


Figure 1. ESI-MS spectrum of the DNA cleavage fragments of the dsDNA (5′-TTAGGGTTAGGG-3′/5′-CCCTAACCCTAA-3′) induced by copper complex of 3-Clip-Phen-Im3-β-Dp, MPA, and air.

$CuCl_2$, and then 1 equiv of the annealed 5′-$(TTAGGG)_2$-3′/5′-$(CCCTAA)_2$-3′ dsDNA was mixed.[21] To this mixture, 10 equiv MPA was added and initiated the DNA cleavage in presence of air. After incubated for 1 h, this mixture was treated with hot piperidine (1 M, 30 min, 90 °C). Before injected to ESI-MS ion source, 20% methanol was added to obtain better spray.[22] ESI-MS spectra were acquired using a LCQ Deca XP Plus ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA), and all the experiments were performed in the negative-ion mode. The spray voltage was 2.0–2.5 kV, capillary temperature 100 °C, and the mixture was introduced into the ESI source at a flow rate of 2 mL min^{-1}.

Figure 1 shows the ESI-MS spectrum of the reaction mixture involving copper complex of 3-Clip-Phen-Im_3-β-Dp and dsDNA (5′-$(TTAGGG)_2$-3′/5′-$(CCCTAA)_2$-3′). In negative-ion-ESI-mass spectra, two characteristic ions of *m*/*z* 1317.5 and 2228.5 were obtained, these two ions indicating directly that the DNA strand is cleaved. The ion at *m*/*z* 1317.5 was found to be 3′-GGGAO-PO_3^-, and the ion at *m*/*z* 2228.5 attaches 5′-TTAGGG $TOPO_3^-$. By analyzing the structures of these two fragments, the cleaving site occurred predominantly at the

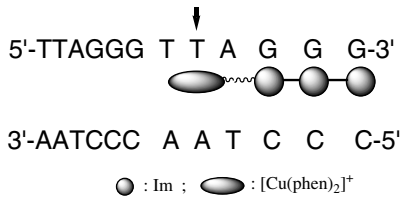
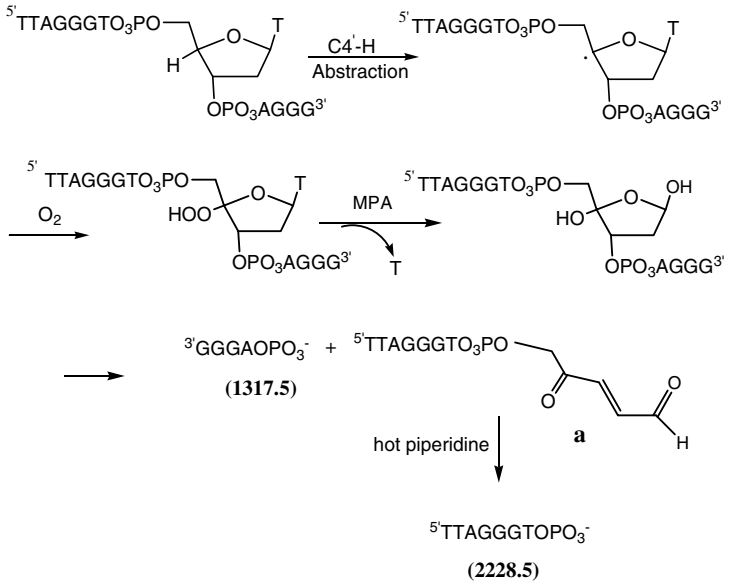


Figure 2. The binding mode and the cleavage site.



Scheme 3. Mechanism of DNA scission.

fourth T moiety of the sequence 5′-TTAGGGTT AGGG-3′. This result shows that the part of polyamide containing three imidazole rings selectively bound three continuous G·C base pairs, and which was consistent with recognization rules about polyamide binding duplex DNA. Figure 2 shows the binding mode that the part of polyamide bound the G·C-rich region of dsDNA (5′-$(TTAGGG)_2$-3′/5′-$(CCCTAA)_2$-3′), and the DNA cleavage agent of copper complex was located at the fourth T base. The mechanism of the site-selective DNA cleavage[23] could be derived from the end group and the existence of the reductant of MPA and air. A possible mechanism for the DNA cleavage[21] is provided in Scheme 3. First, the radical produced by the copper complex and MPA abstracted a hydrogen atom at the 4′-carbon to afford the deoxyribose-center radical. This latter radical combined with the oxygen in air to give its peroxide. Under the reduction of excess MPA, this peroxide was reduced to 4′-hydroxyl deoxyribose. Then, the latter discharged a free base of T, 3′-$GGGAOPO_3^-$ (*m*/*z* 1317.5), and fragment with 3-phospho-keto-aldehyde termini (**a**) through β-elimination. Compound **a** was further degraded to 5′-$TTAGGGTOPO_3^-$ (*m*/*z* 2228.5) under the treatment of hot piperidine.

In this research, 3-Clip-Phen-Im_3-β-Dp was successfully synthesized for the targeting 5′-$(TTAGGG)_2$-3′/5′-$(CCCTAA)_2$-3′ DNA, and the site-selective cleavage of the complex of copper-conjugate was confirmed by the analysis of ESI-mass spectra of the cleavage product. This result shows that ESI-MS is a very useful tool to evaluate the behavior of selective DNA cleaving.

$NO_2ImImIm\beta Dp$. To a solution of NO_2ImImImβCOOH (0.50 g, 1.00 mmol) in 8 mL of anhydrous DMF were added HOBT (0.14 g, 1.00 mmol) and DCC (0.21 g, 1.00 mmol). After the reaction mixture was stirred for 12 h, *N*,*N*-dimethylpropyldiamine (120 μL, 1.0 mmol) was added. The mixture was stirred for 12 h, and then the solvent of DMF was evaporated in vacuo. The residue was purified by flash column chromatography with $CHCl_3/CH_3OH$ (1:2, v/v) to afford the product (0.27 g, 48% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.32 (s, 1H), 8.09 (s, 1H), 8.11 (m, 1H), 7.50 (s, 1H), 7.49 (s, 1H), 7.24 (s, 1H), 7. 22 (m, 1H), 4.25 (s, 3H), 4.07 (s, 3H), 4.01 (s, 3H), 3.68 (q, 2H), 3.35 (q, 2H), 2.51 (m, 4H), 2.32 (s, 6H), 1.74 (m, 2H); ESI-MS *m*/*z* 573 (MH^+, $C_{23}H_{33}N_{12}O_6$).

3-Phen-clip-ImImImβDp. To a solution of 3-Phen-clip-COOH (40 mg, 0.07 mmol) in 1 mL of anhydrous DMF were added HOBT(9 mg, 0.07 mmol) and DCC (15 mg, 0.07 mmol). The reaction solution was stirred for 12 h. Separately, to a solution of NO_2ImImImβDp (45 mg, 0.07 mmol) in 2 mL DMF was added Pd/C catalyst (10%, 6.0 mg), and the mixture was stirred under a positive pressure of H_2 overnight. Then catalyst was removed by filtration through Celite, and the filtrate was directly added to the solution of active ester. After stirred for 12 h, DMF was evaporated in vacuo. The residue was purified by flash column chromatography with $CHCl_3/CH_3OH$ (1:2, v/v) to afford the yellow solid (25 mg, 45% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.65 (s, 1H), 9.13 (d, 2H, J = 4.4 Hz), 8.83 (d, 2 H, J = 4.0 Hz), 8.65 (s, 1H), 8.54 (d, 1H, J = 4.0 Hz), 8.44 (d, 2 H, J = 7.2 Hz), 8.37 (s, 1H), 8.25 (m, 1H), 8.17 (d, 2H, J = 4.2 Hz), 8.08–7.96 (m, 5H), 7.72–7.67 (m, 4H), 7.53 (s, 1H), 4.73 (m, 1H), 4.48 (m, 4H), 4.06 (s, 3H), 4.03 (s, 3H), 3.96 (s, 3H), 3.44 (m, 4H), 3.12 (m, 2H), 2.46–2.36 (m, 6H), 2.29 (s, 6H), 1.64 (m, 2H); ESI-MS *m*/*z* 1072 (MH^+, $C_{54}H_{58}N_{17}O_8$).

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